4. HIGH TEMPERATURE EFFECT ON THE SPORES OF USTILAGO MAYDIS (DE CANDOLLE) CORDA

M. KEDVES and A. TÓTH

Cell Biological and Evolutionary Micropaleontological Laboratory of the Department of Botany of the J. A. University, H-6701, P. O. Box 657, Szeged, Hungary

Abstract

This paper summarizes the first results about the experimental studies of the teliospores of *Ustilago maydis*. The high temperature effect to the spores was investigated in detail with the LM method. Temperature: 200 °C, length of time from 10′ until 300 hrs. On the basis of the first results we have established as follows. 1. During the heating of the teliospores the outer sculptured part of the wall is destroyed. This starts after 10 hours′ heating, and is extremely advanced at the length of time of 100 hours. 2. The outermost wall layers lost spores, mounted in glycerine-jelly form interesting patterns, which are very useful in the modelling of the biopolymer structures of the partially degraded plant cell wall. 3. As regards the variation-statistical graphs of the diameter of the spores, several anomalies were observed. Some of them are in connection with the degradation of the wall, but there are also problems to be solved in the future.

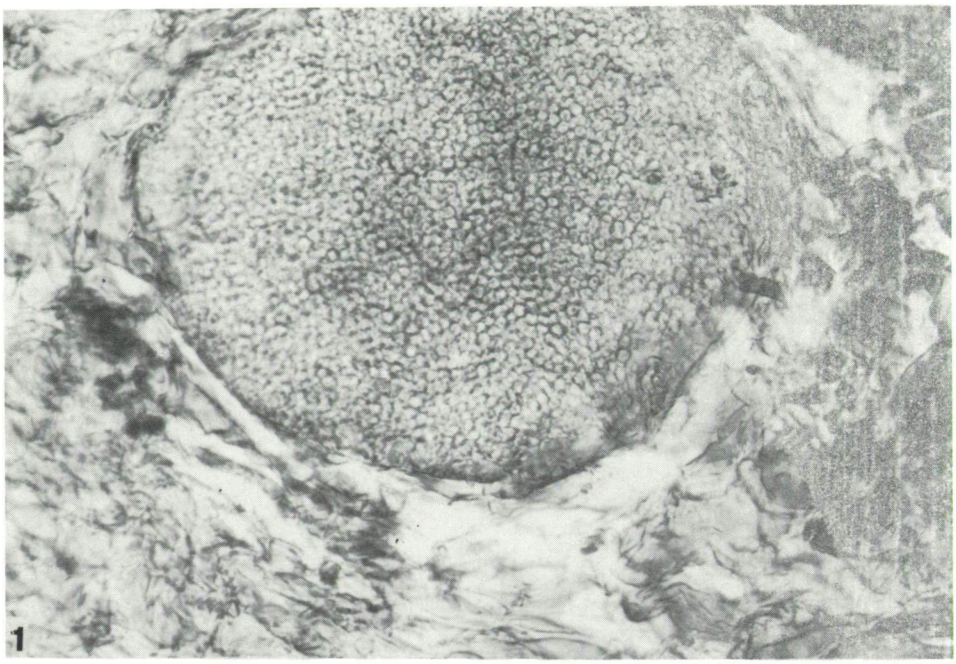
Key words: Palynology, recent, *Ustilago,* high temperature effect.

Introduction

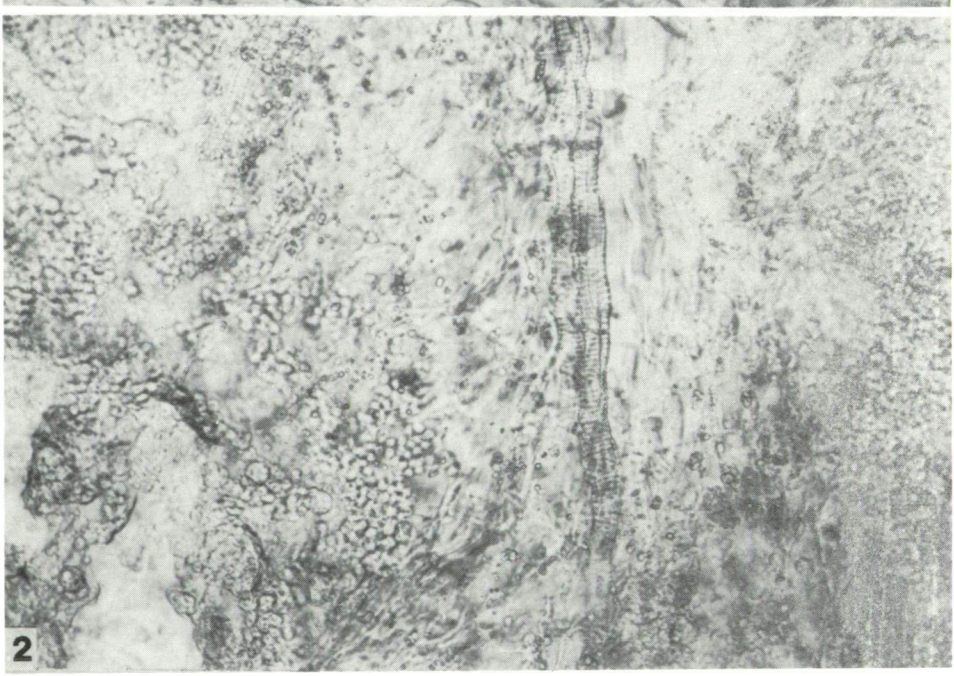
In the last years the research program of the laboratory has changed and been completed with the new fields of researches. From the new fields several papers were also published; nearly from the biopolymer organization and symmetry of the plant cell wall, and about the experimental investigations on the spores and pollen grains. Until this time mostly spores of *Pteridophyta* and pollen grains *(Gymnospermatophyta, Angiospermatophyta)* were the subject of our experimental studies. But the extremely resistant wall of the spores and cysts of the parasitical microorganisms was also planned with the biopolymer structure of the animal cyst shells and cuticles. This paper presents the first results on this subject of the laboratory.

Plate 4.1. ▶

1,2. *Ustilago maydis* (DE CANDOLLE) CORDA, Recent.
LM pictures from sections of infected corn of mayze. 250x.
1. Spore ball in the infected corn.
2. General aspect from the infected corn tissue with parenchymatous and tracheary (secondary wood) elements.



1



2

Materials and Methods

The investigated material was collected by Dr. A. PALÁGYI on 22. 8. 1991. Locality: Ságvári Experimental Research Station of the Cereal Research Institute. The spores were frozen at – 20 °C after collection. The high temperature effect at 200 °C on the air dried spores was made as follows.

Length of time	Experiment No
0′	1192
10′	1193
1^{h}	1194
5^{h}	1195
10^{h}	1196
25^{h}	1197
50^{h}	1198
100^{h}	1199
200^{h}	1200
300^{h}	1201

The slides for light-microscopical investigations were mounted in glycerine-jelly hydrated at 39.6%. 200 specimens of each sample were investigated. To get a general aspect about the anatomy of the infected tissues of the corn of maize sections for LM investigations were made (by Mr. L. TÓTH–SOMA). The thin sections were stained with Safranine T, Bismarck Brown, and Toluidine blue and investigated with the LM method. The pictures were taken with an objective Carl Zeiss Jena, GH Planachromat 40X/0.65/0.17-A, (Plate 4.1., fig. 1,2), respectively with an objective of oil immersion Carl Zeiss Jena, GF Planachromat HI 100X/1.25/0.17-A (Plate 4.2. – plate 4–5.).

Halfthin and ultrathin sections were also made for TEM investigations. At this moment the aim of these preliminary studies was to get a basis for the fine structure and ornamentation of the wall. The transmission electron microscopy of the partially degraded spore wall and the investigation of the biopolymer system will be the subject of further investigations.

As regards the nomenclature and the previous data about the spores of *Ustilago maydis* (DE CANDOLLE) CORDA as basic works the following monographs were used: BÁNHEGYI, TÓTH, UBRIZSY and VÖRÖS (1985), VÁNKY (1985). As regards the LM measurements interesting methods by KÖHLER, 1933 and MECKE 1920 were published in the monograph of ERDTMAN (1954). SEM data: cf. VÁNKY, p. 222, Fig. 213 bis. Morphology of the fresh spores after the work of VÁNKI (1985), p. 222: "Spores globose, subglobose, ovoid to sometimes elongated or slightly irregular, 7–11×7–13 μm, light olive-brown; wall c. 0.5 μm thick, finely, rather densely echinulate. Germination by four-celled promycelium laterally and terminally bearing basidiospores."

Results

1. HISTOLOGY OF THE INFECTED CORN OF MAIZE (Plate 4.1., figs. 1,2, plate 4.2.)

These investigations are of a character of orientation. Illustrated are the not completely mature *Ustilago maydis* teliospores in the blasted corn. The delimination of the spore ball and the altered cells of the parenchymatous tissues (Plate 4.1., fig. 1, plate 4.2.) are well shown. In Plate 4.2. the small size and the hexagonal ambitus of the immature spores are illustrated. Picture 2 (Plate 4.1.) illustrates the dispersion of the infection with secondary xylem elements, tracheas.

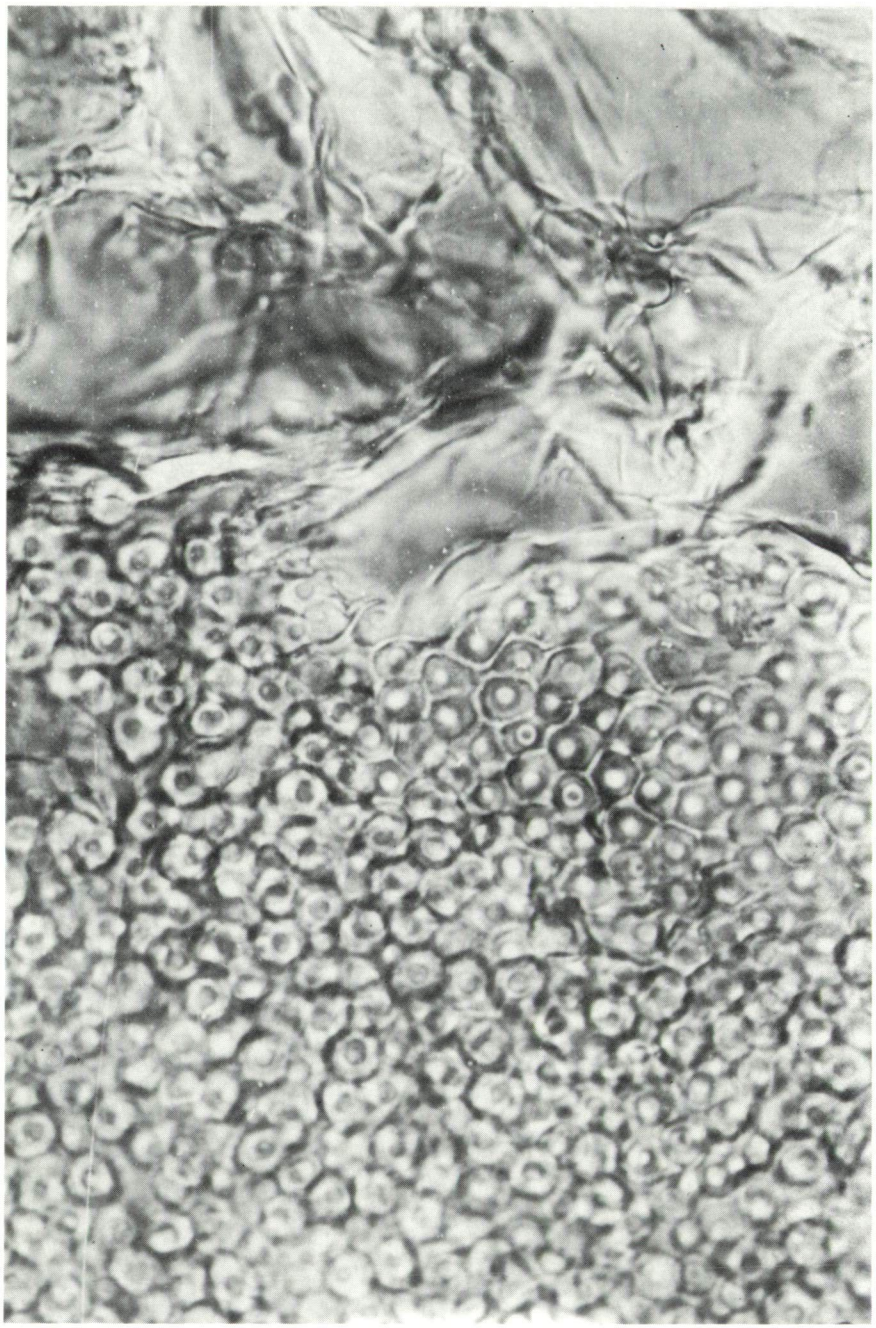
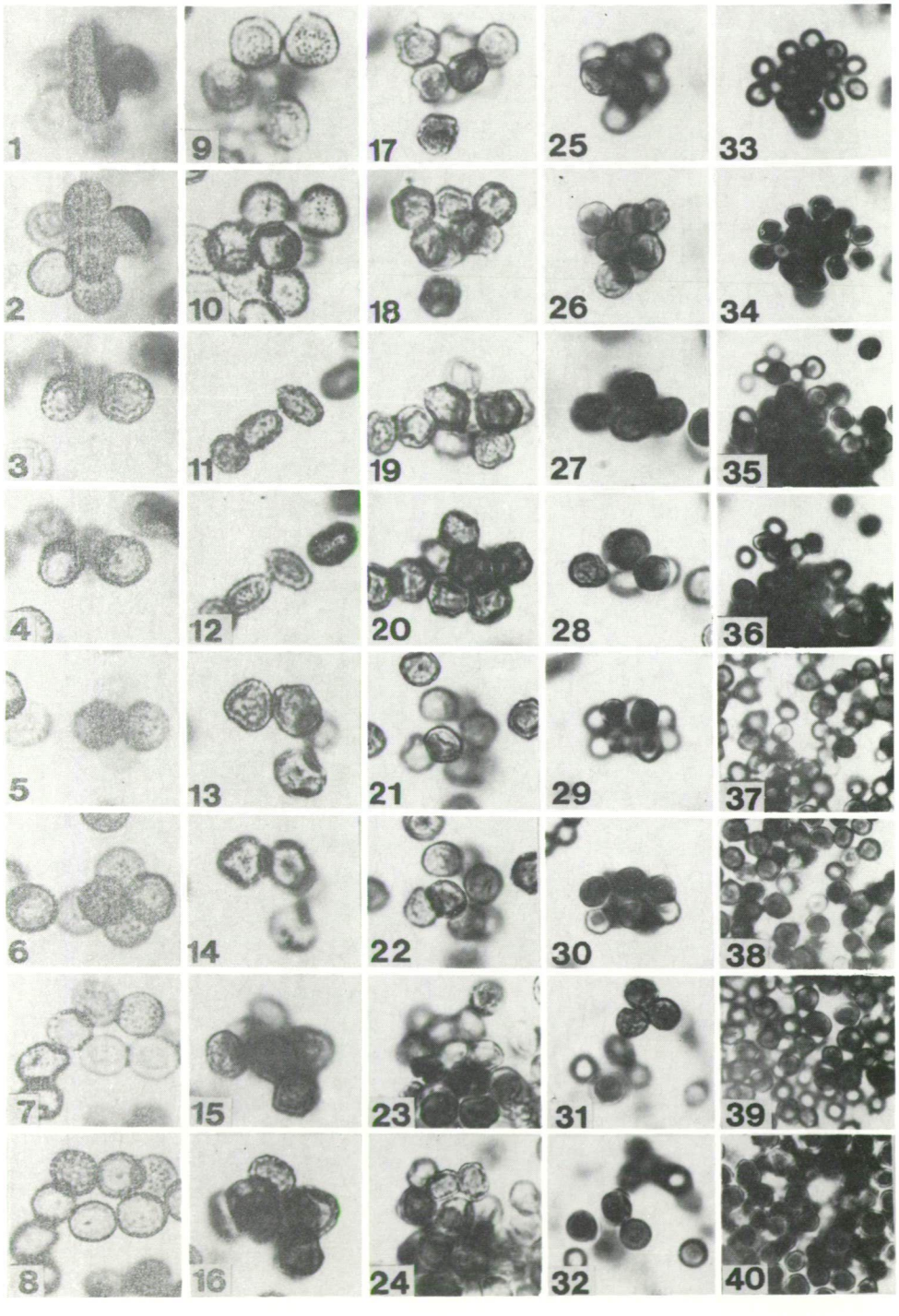


Plate 4.2.

Ustilago maydis (De Candolle) Corda, Recent.
LM picture from the detail of the bordering of the spore ball in the parenchymatous tissue. The angular ambitus of the immature spores is characteristic. 1000×.


1
9
17
25
33
2
10
18
26
34
3
11
19
27
35
4
12
20
28
36
5
13
21
29
37
6
14
22
30
38
7
15
23
31
39
8
16
24
32
40

◄ Plate 4.3.

1—40. *Ustilago maydis* (DE CANDOLLE) CORDA, Recent.
1—4. Spores without staining or heating. Experiment No 1192.
5—8. Experiment No 1193, length of time 10'.
9—12. Experiment No 1194, length of time 1 hr.
13—16. Experiment No 1195, length of time 5 hrs.
17—20. Experiment No 1196, length of time 10 hrs.
21—24. Experiment No 1197, length of time 25 hrs.
25—28. Experiment No 1198, length of time 50 hrs.
29—32. Experiment No 1199, length of time 100 hrs.
33—36. Experiment No 1200, length of time 200 hrs.
37—40. Experiment No 1201, length of time 300 hrs.
1000×.

2. THERMAL ALTERATIONS OF THE MATURE TELIOSPORES
(Plate 4.3., figs. 1—40, plate 4.4 figs. 1—3, plate 4.5., figs. 1—7, text-figs. 4.1., 4.2., and 4.3.)

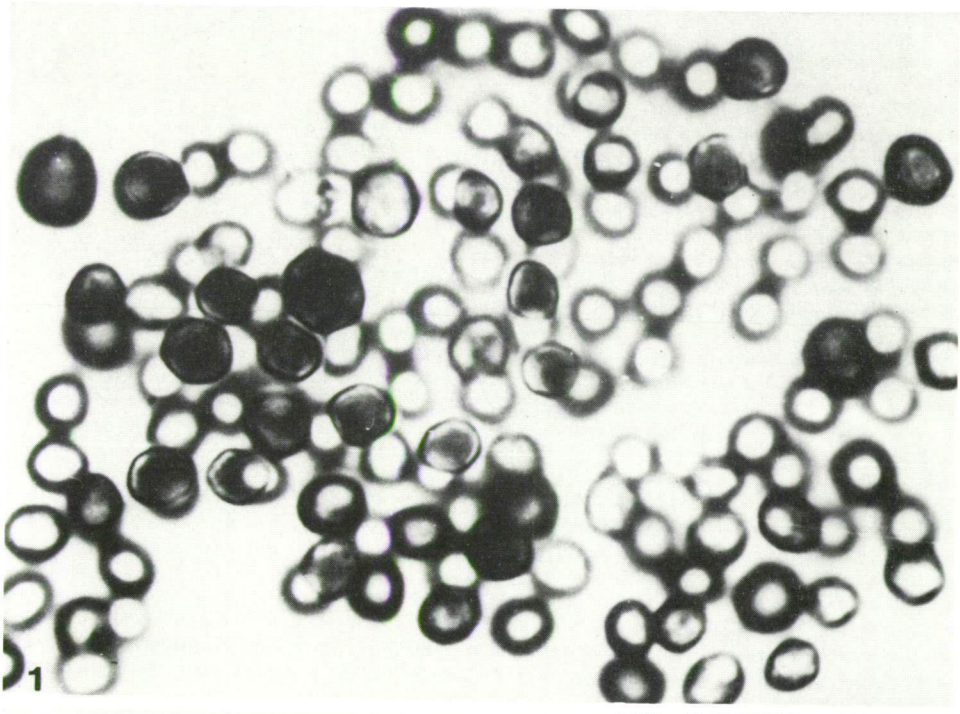
As regards the general morphology of the mature spores, our observations are concordant with the description of VÁNKY (1985, p. 222), mentioned previously.

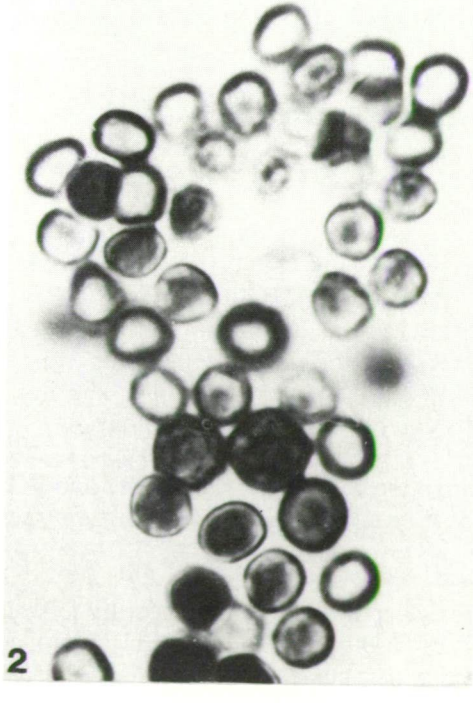
2.1. *Qualitative alterations of the spores in consequence of the high temperature*
(Plate 4.3., figs. 1—40)

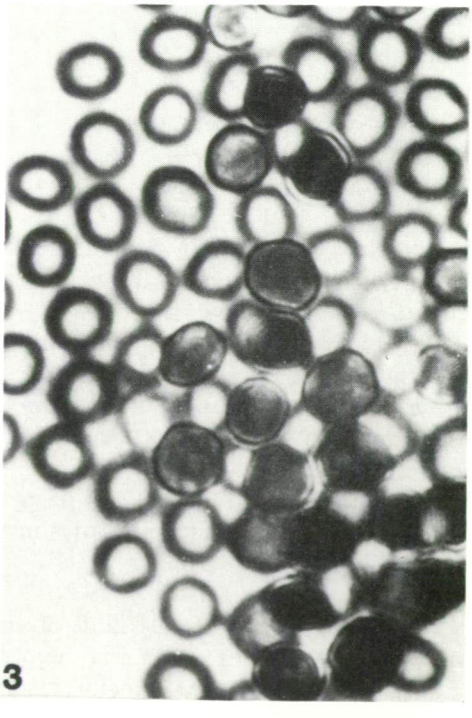
Two important degrees of the secondary alterations can be established on the basis of the spore wall structure;
i. alterations with a complete spore wall,
ii. alterations of the outer wall lost spores.
At the first step of alteration the shape is in some cases elongated (Plate 4.3., figs. 11,12), and the ambitus can be newly hexagonal, a little similar to the immature spores (Plate 4.3., figs. 13—20). After 10 hours of heating at 200 °C the destruction of the spore wall starts. Parallel to this alteration the secondarily smooth (non sculptured) spores have newly globular or nearly globular form. These are interesting and unusual, in contrast to the previous ones. The most important characteristic features and results can be summarize as follows.

1. In Plate 4.3., figs. 27—40 and particular in the pictures of Plate 4.4. and 4.5. it is well shown that there are two kinds of spores. The darker are a little larger than the light coloured ones.

2. The arrangement of this kind of spores after mounting in glycerine-jelly is interesting, and these patterns seem to be useful to understand the space arrangement of the biopolymer structures, in particular of the quasi-crystalloid and quasi-equivalent systems.


1


2


3

◄ Plate 4.4.

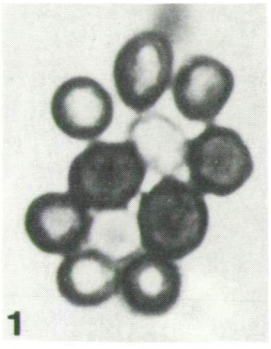
1—3. *Ustilago maydis* (DE CANDOLLE) CORDA, Recent.
Experiment No 1201, outer sculptured wall-layer lost spores mounted in glycerine-jelly, forming interesting patterns. 2.500×.

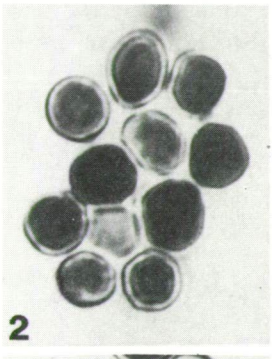
Linear arrangement. — The outer-layer lost globular spores can be arranged into one or two lines. The one cell composed lines can be divided. 64°, 70° and 90° were measured as angle of the bifurcate line (Plate 4.4., fig. 1). Alternating arrangements have been observed at the spore pattern of two lines (Plate 4.5., fig. 3,4).
Pentagonal and hexagonal arrangements of this kind of spores were also observed (Plate 4.4, fig. 2). Particularly interesting pentagonal "spore modell" can be seen in picture 3. Plate 4.4. The connection of the regular pentagonal arrangement with a hexagonal one (Plate 4.5., fig. 1,2) is a part of the model of the quasi-equivalent system.
Text-fig. 4.1., and 4.2. summarize the schemas which were made from the LM pictures of these spores.

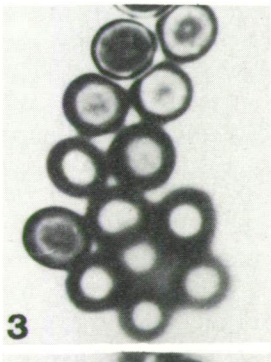
2.2. *Quantitative alterations of the spores in consequence of the high temperature* (Plate 4.3., figs. 1—40, text-fig. 4.3.)

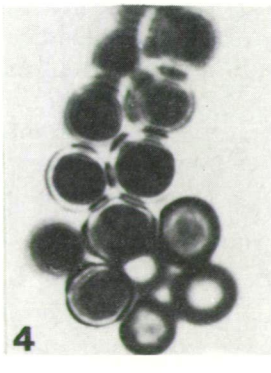
The variation-statistical graph of the intact spores is peculiar (Text-fig. 4.3., A, B,). 10′ and 1^h of heating resulted nearly the same graph with maximum. The graph of the spores heated for 5 hours is almost the mirror image of those of the intact spores. The proportions of the diameter of the spores heated for 10 hours are extremely interesting. The variation-statistical graph of the spores heated for 25 and 50 hours represents well the regular diminution in size of the spores. In contrast to this the variation-statistical graph of the spores heated for 100 hours is different and the most interesting. Namely this graph forms a regular shape of "V" with the graph of the intact spores. This can be taken as an extreme dislocation of the variation-statistical graphs of the non-experimental and experimental spores. The spores heated for 200 and 300 hours resulted in nearly the same diminution. It is noteworthy that the maximum size (7.5 μm) is identical with the smallest spores of the non experimental spores and those of heated for 10′, 1 hour 5, and 10 hours.

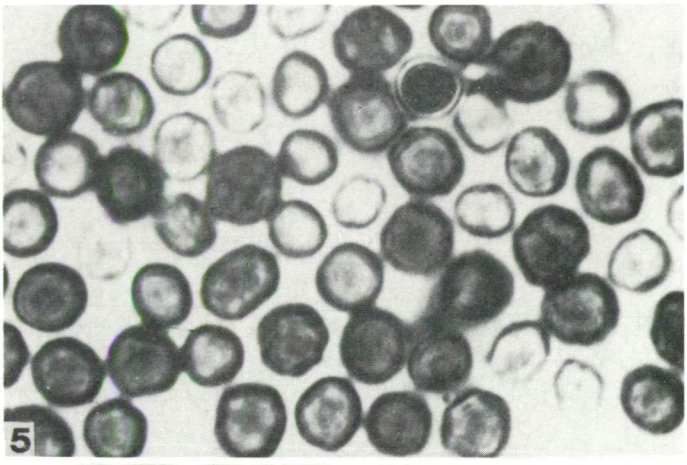
The ratio of the longest and smallest size (Text-fig. 5.3., C, D) represents approximatively regular alterations. Extreme graphs such as pointed out previously cannot be stressed.

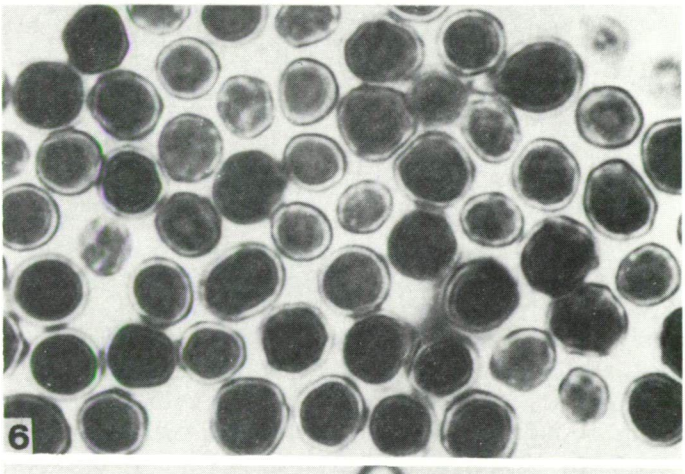

1

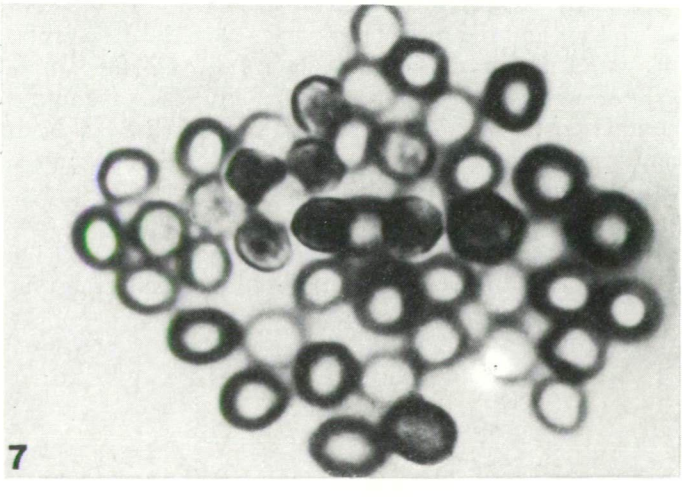

2


3


4


5


6


7

◀ Plate 4.5.

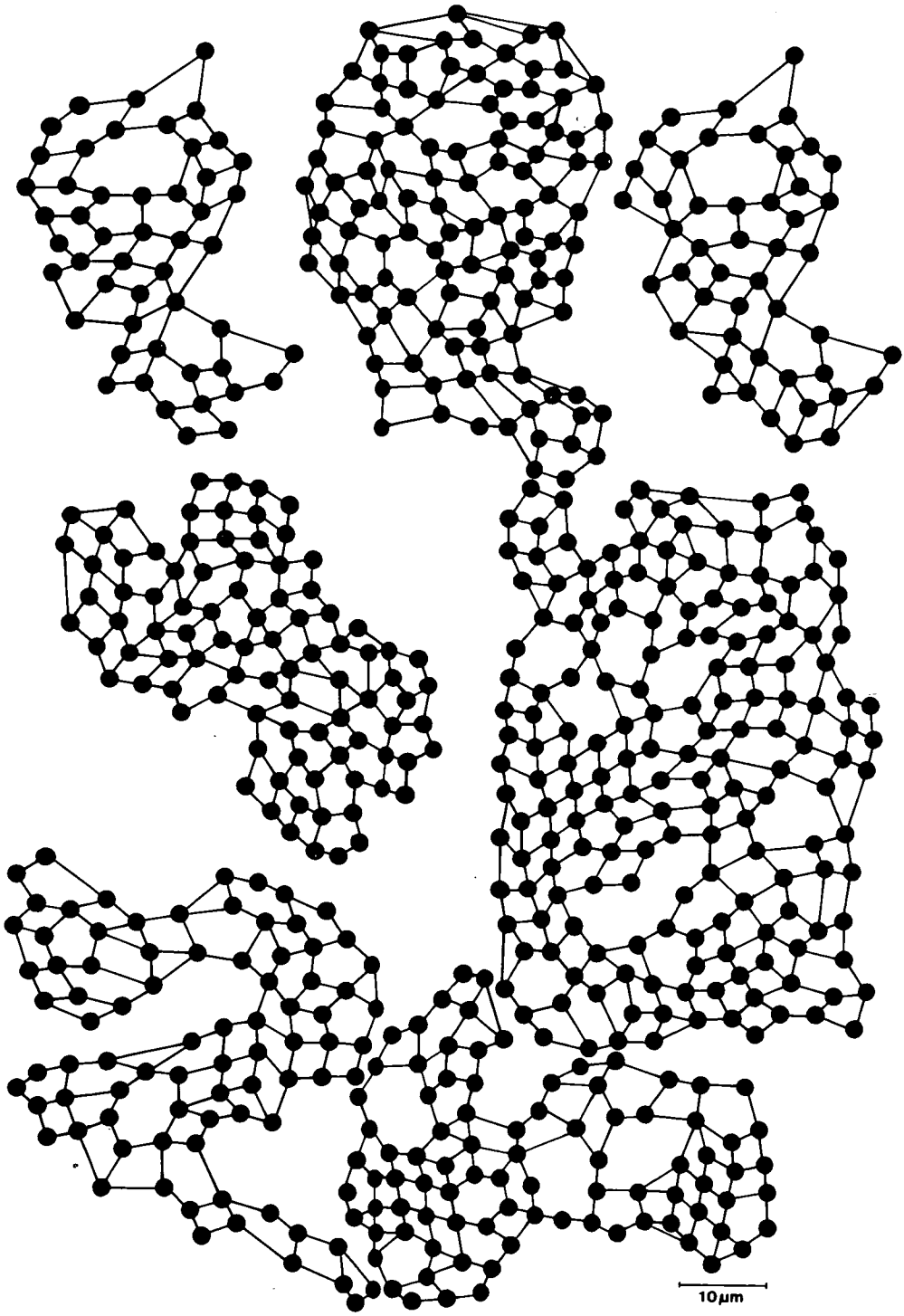
1—7. *Ustilago maydis* (De Candolle) Corda, Recent.
Experiment No 1201, outer sculptured wall-layer lost spores mounted in glycerine-jelly, forming interesting patterns. 2.500×.

1,2. Pentagonal; and hexagonal arrangement.

3,4. Pentagonal arrangement connected with alternate linearly aligned spores.

5,6. General aspect of the spore pattern. The heterogeneous character of these spores, the darker and larger, and the light-coloured smaller ones are shown well.

7. A part of the massula of spores of different kinds of arrangement.


10µm

◄ Text-fig. 4.1.

Schemas made from the LM pictures of the pattern of the arrangement of the spores after experiment No 1201.

Discussion and Conclusions

1. It is necessary to emphasize that the results, presented in this paper represent our first attempts in this field. We hope that this will be followed by several further ones.

2. The "anomalies" in the variation-statistical graphs, particulary in the largest diameter of the spores must be later investigated as well. The degradation of the outer spore wall layers starts approximatively after 10 hours of heating. But it seems that this fact cannot explain the peculiarity of this variation-statistical graph. But the most interesting, and important anomaly of the variation-statistical graph was observed at spores heated for 100 hours. This is all probability in connection with the extremely advanced degradation of the outest sculptured layers of the spore wall.

3. The peculiar arrangement and the pattern of the outer wall layer lost spores can be taken as a peculiar kind of modelling of the biopolymer structures, composed from globular basic units.

4. There are some similarities between the sculptural layer lost teliospores and some fossil forms observed and illustrated in the sediments, in particular in the metamorphic layers.

Acknowledgements

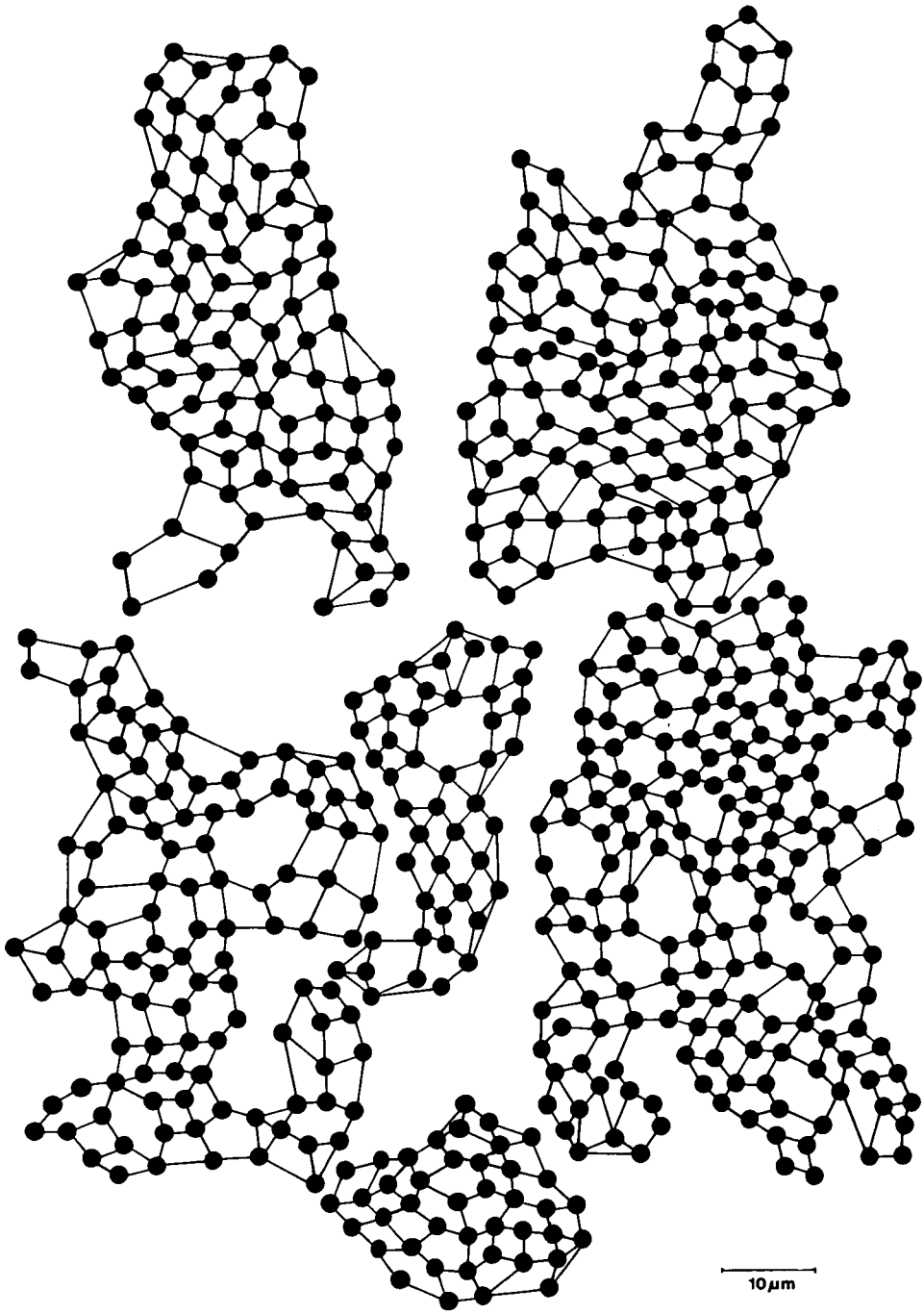
This work was supported by the grant OTKA 1/3, 104. The authors are deeply indebted to Mr. L. TÓTH—SOMA for preparing the sections for LM investigation from the infected corn of mayze.

References

BÁNHEGYI, J., TÓTH, S., UBRIZSY, G. and VÖRÖS, J. (1985): Magyarország mikroszkópos gombáinak határozókönyve 2. kötet *Eumycotina (Ascomycetes:* A *Discomycetes*től, *Basidiomycetes, Deuteromycetes)*. — Akadémiai Kiadó, Budapest.

ERDTMAN, G. (1954): An Introduction to Pollen Analysis. — Almqvist and Wiksell, Stockholm.

VÁNKY, K. (1985): Carpathian *Ustilaginales*. — Acta Univ. Upsaliensis, Symbolae Botanicae Upsaliensis *24*, 1—309.



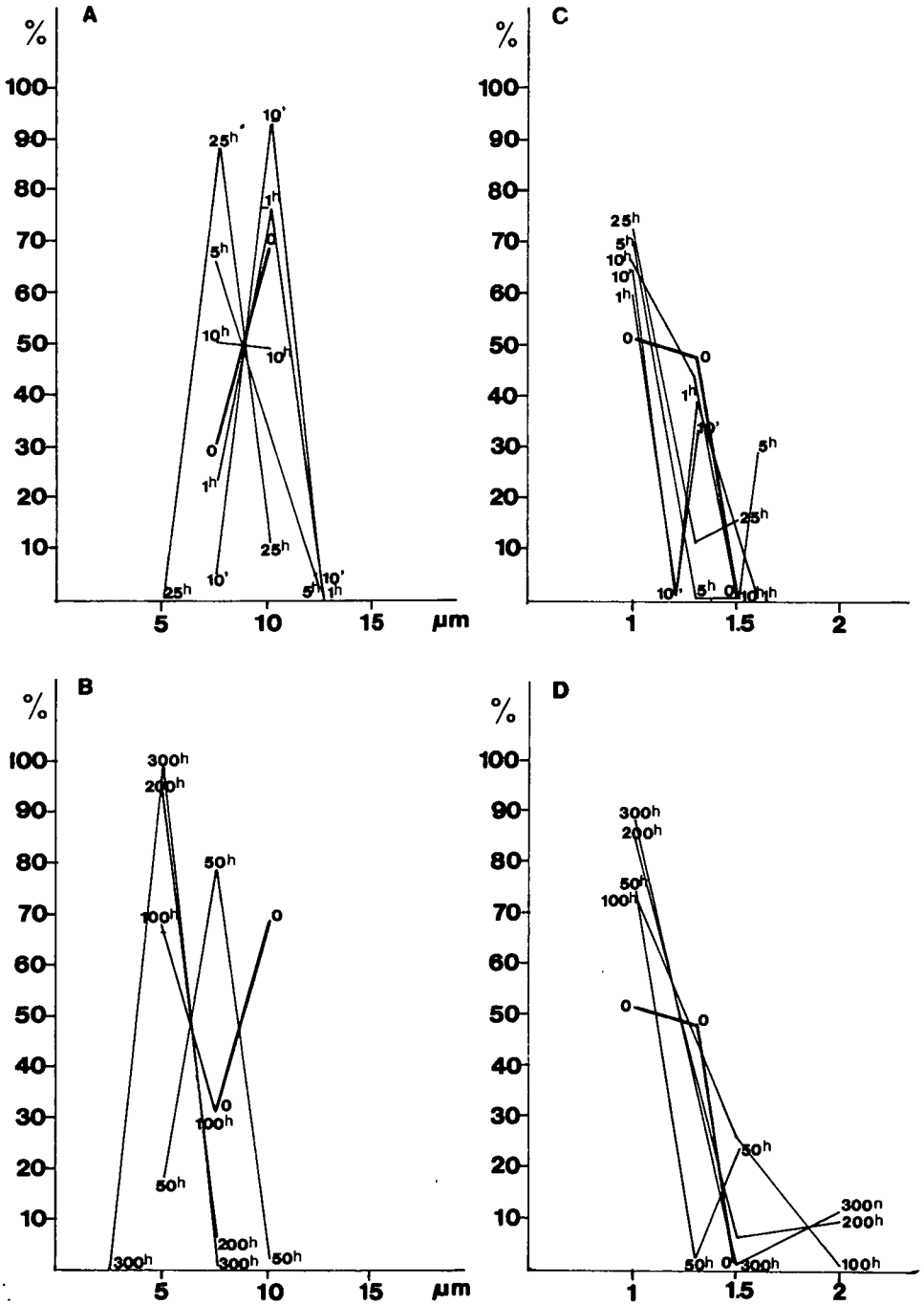
Text-fig. 4.2.

Schemas made from the LM pictures of the pattern of the arrangement of the spores after experiment No 1201.

Text-fig. 4.3. ▶

A,B. Variation-statistical graphs of the alternations of the maximum diameter of the spores in consequence of high temperature. The numbers at the graphs indicate the length of times of heating at 200 °C.

C,D. Variation-statistical graphs of the alternation of the longest and smallest diameter of the spores.


A
%
100
90
80
70
60
50
40
30
20
10
5
10
15
µm
25h
10'
1h
0
5h
10h
B
300h
200h
50h
100h
C
1
1.5
2
D